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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	09/771,277
Applicant	:	José A. Olivares et al.
Title	:	PARTICLE SIZER AND DNA SEQUENCER
Filed	:	January 26, 2001
TC/A.U.	:	1753
Examiner	:	John S. Starsiak
Docket No.	:	4250.2.6
Customer No.	:	21552

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.131

Dear Sir:

We, José A. Olivares and Peter C. Stark, hereby declare as follows:

1. We are coinventors of the subject matter described and claimed in the above-identified patent application.

2. Prior to July 6, 2000, we conceived and reduced to practice a working embodiment of the invention claimed in the above-identified patent application. Prior to July 6, 2000, we prepared an Invention Disclosure describing the system as shown in Figures 1 and 2 of the above-identified patent application. The Invention Disclosure documents how the system successfully worked for its intended purpose prior to July 6, 2000. Attached as Exhibit A are pages 1-3 and 9 of the Invention Disclosure as well as pages 51, 59, 60, and 70 of José A. Olivares' laboratory notebook that were attached to the Invention Disclosure. Attached as Exhibit B is a transmittal form of the Invention Disclosure and a second Invention Disclosure that was prepared prior to July 6, 2000. These acts all occurred in the United States.

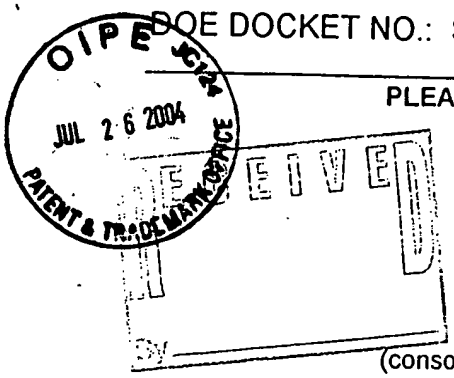
3. Each of the dates deleted from Exhibit A and Exhibit B is prior to July 6, 2000.

4. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: July 15, 2004 _____
José A. Olivares

Dated: 7.15.04 _____
Peter C. Stark

S:\ALLCLIENTS\4250 Los Alamos\4250.2.06\4250.2.6 131 Declaration.doc



DOE DOCKET NO.: S-_____ LAD-99-109

PLEASE DO NOT COMPLETE ANY INFORMATION ABOVE THIS LINE

UNIVERSITY OF CALIFORNIA
THE LOS ALAMOS NATIONAL LABORATORY
LOS ALAMOS, NM 87545

INVENTION DISCLOSURE

(consolidated Record of Invention and Invention Evaluation Questionnaire)

This invention was made in the course of or under prime Contract No. W-7405-ENG-36 between the U.S. Department of Energy and the Regents of the University of California. This Invention Questionnaire is an important legal document and should be fully and carefully prepared in accordance with the following instructions.

INSTRUCTIONS: 1) This Invention Disclosure will form the basis from which UC will determine whether to elect title to this invention and proceed to seek patent protection. It is important that you provide as much information as possible. 2) Please submit **completed** Disclosure to the Intellectual Property Management (IPM) team within the Civilian and Industrial Technologies Program Office (CIT-PO), MS C334. 3) The appropriate Group Leader(s) **must** sign the completed Disclosure before it is submitted for review.

If you have any questions, please call the Annabelle Torres at 667-8129 or Sharon Trujillo at 665-6708. IPM will coordinate the patent filing decision with Laboratory Counsel, Business and Patent Law (LC/BPL) and the appropriate Capability Access Team (CAT), and will contact you once this decision has been made. The answers to the following questions will be reviewed by the appropriate CAT and by LC/BPL. You may be contacted by LC/BPL or the CAT for additional information. Both will use this information to determine if a patent application will be filed on behalf of the University of California. Your answers should be in non-technical language as they will form the basis of this business decision.

Source of Funding (Program or Agency): Low Dose Radiation Program

☐ CRADA ☐ User Facility ☐ Technical Assistance
☐ Work For Others ☐ LDRD ☐ Other _____

DOE Program Director: SCOTT CRAM

DOE B&R Code: KP1102O2

Please provide input regarding the category this invention best represents.
(Refer to the Capability Access Team Reference Guide for further details)

THIS DOC IENT CONTAINS PRIVILEGED I: ORMATION

Check only ONE: ☐ Materials ☐ Computing ☐ Chemistry
☒ Bioscience ☐ Engineering & Physical Science

Invention Information

1. **Title of Invention** (indicate briefly the name of the article, device, material, composition, or process)

Protein & DNA fragment sizer & sequencer

2. **Discloser(s):** The list should include all individuals who are believed to have made an original contribution to the inventive concept and a substantial contribution to its reduction to practice. When in doubt, it is best to include a person rather than exclude a person. The final determination of inventorship will be made by LC/BPL after the invention is defined and after discussion with the disclosers listed below.

Name	MS	Phone	Home Address	Employer	Z#
JOSE A. OLIVARES	J565	55190	361 KIMBERLY LOS ALAMOS, NM	LANL	104550
PETER STARK	J565	70724	LOS ALAMOS, NM	LANL	120536

3. **Attach a description of the Invention.** Include as many pages and attachments as needed to fully describe your invention, and how it differs from the state of the art, including any experimental protocols and results. You should also attach copies of notebook pages and other written documents that are pertinent to the invention.

Suggested Format:

A. Brief non-technical abstract of the Invention
B. Background of the Invention, including a statement of the problem(s) to be overcome and previous attempts to solve these problems (include reference materials on the problem(s) and the attempted solution(s)).
C. Statement of Invention (what did you invent and what are the advantages)
D. Detailed description of the Invention (include drawings, photos, graphs, etc.) in sufficient technical detail for the reader to understand the invention.

4. **Dates and Places of Invention:**

a) **Conception of Invention:** _____ (date) at Los Alamos National Lab (where)

(Give the earliest date on which, and the place where, the invention was suggested, even if not complete. If the invention includes several inventive concepts, give the conception date of each and clearly identify the contributor(s) of each element).

b) **First Sketch or Drawing:** _____ (date) at LANL (place) in Notebook JA0-001 Page 51 (number)

(Give the date of the earliest record that is available)

c) **First Written Description:** _____ (date) at LANL (where) in Notebook JA0-00/ Page 70 & 71 (number) 51

(Give the date of the earliest record that is available)

d) **Completion of Model or Full Size Device:** _____ (date) at LANL (where)

first successful

e) **First Test or Operation of Invention:** _____ (date) at LANL (where)

Degree of success attained (List successive dates if successive results are available)

One capillary system demonstrated

Multi capillary system is an extension

5. a) **What is the present stage of development of this Invention?** (Please check one)

☐ Concept (A bare idea with sufficient thought to provide initial direction toward a reduction to practice)

☒ Bench Design (An initial test of a complete Invention using laboratory resources; not engineered)

☐ Lab Prototype (An engineered design that incorporates the complete Invention, but not engineered to use in its intended environment)

☐ Lab Testing (Sufficient testing to obtain proof-of-principle verification)

☐ Field Prototype (An engineered design that may be used outside the laboratory in its intended environment)

☐ Ready for Transfer (An engineered and tested process or equipment with test results to demonstrate the capabilities of the Invention)

b) **Have you achieved "actual" reduction to practice?** (i.e. did you achieve the desired result -- operating machine, desired material, process control -- in accordance with the description of the Invention provided above)

18. **Have any commercial entities expressed an interest in this technology?**
(If the company's interest in this Invention was for government use only, please state)
Yes_____ No X If Yes, please list the companies and describe their interest.

_____

_____

_____

_____

_____

_____

19. **This Invention Questionnaire was completed by:**

Name	Position/Title	Date
JOSE A. OLIVARES	STAFF MEMBER	

Discloser(s)/Line Manager Signature(s)

20. **Each discloser needs to sign and date the Invention Questionnaire.** If license income is generated as a result of this Invention, a portion of the income is returned to the division. Therefore, it is necessary to identify the Division to which the discloser(s) was(were) assigned at the time that 1) the Invention was conceived or first reduced to practice, 2) the software or other copyrighted work was authored, or 3) the mask work was created.

The line manager of the discloser(s) must review the Invention Questionnaire and sign-off indicating that he/she believes the technology to be sound and recommends that the University of California should seek patent protection.

I/We have reviewed this Invention Questionnaire and recommend that it be considered for a patent application:

JOSE A. OLIVARES, CST-9

NOTEBOOK # JAO-001

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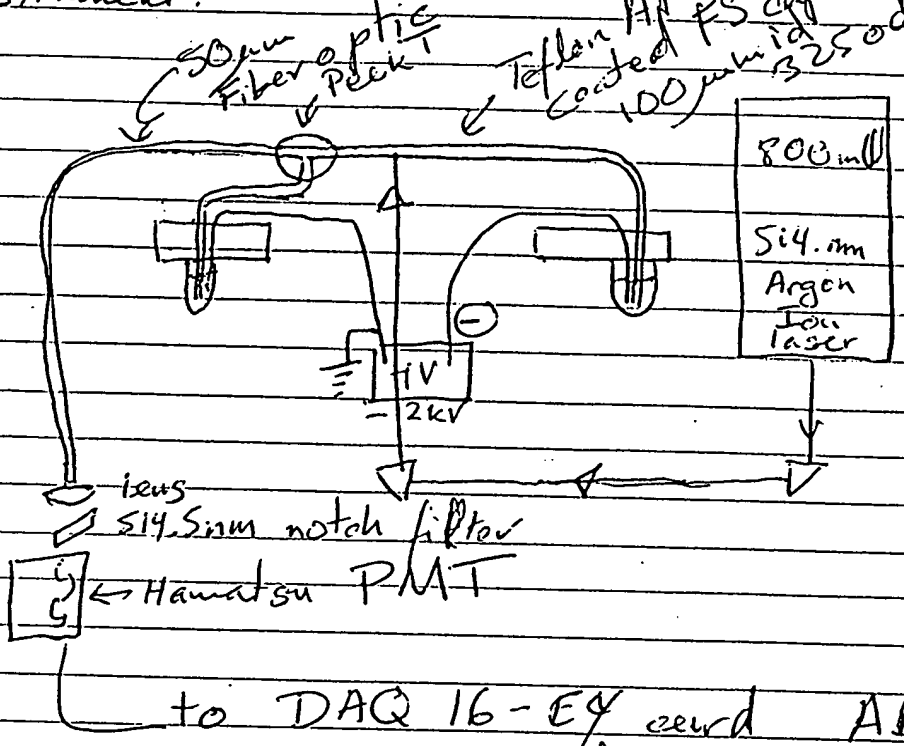
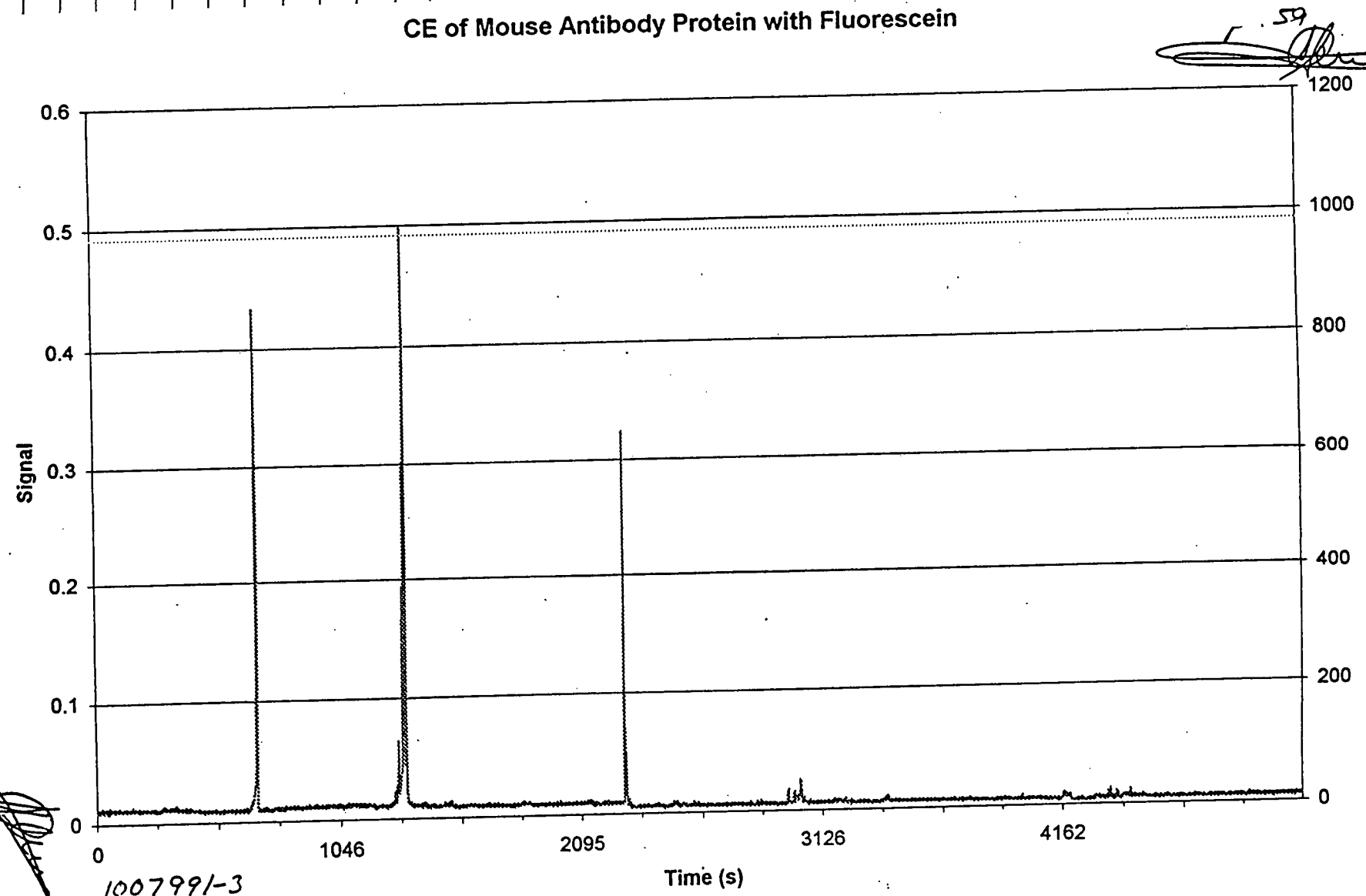
DNA Oxydative Damage Capillary Electrophoresis Assay System

Instrument:



- Teflon coating has index of refraction < water instead of polyimide
- laser illuminates waveguide and beads
- fluorescence from each band
- Resolution of our system can be selected by changing slit width of fixed window
- Can collect more than one band

Not used


CE of Mouse Antibody Protein with Fluorescein
Signal
0.6
0.5
0.4
0.3
0.2
0.1
0
1200
1000
800
600
400
200
0
0
1046
2095
3126
4162
Time (s)

1007991-3

Laser background signal current with capillary position
1 Inch steps (0 closest to FO)

Laser position	Start (sec)	Stop	file
0	200	250	1008991
1	450	500	↓
2	600	650	
3	5	53	
4	775	825	
5	975	1025	↓
6	150	200	1008992
7	275	325	↓
background	100	150	1008991

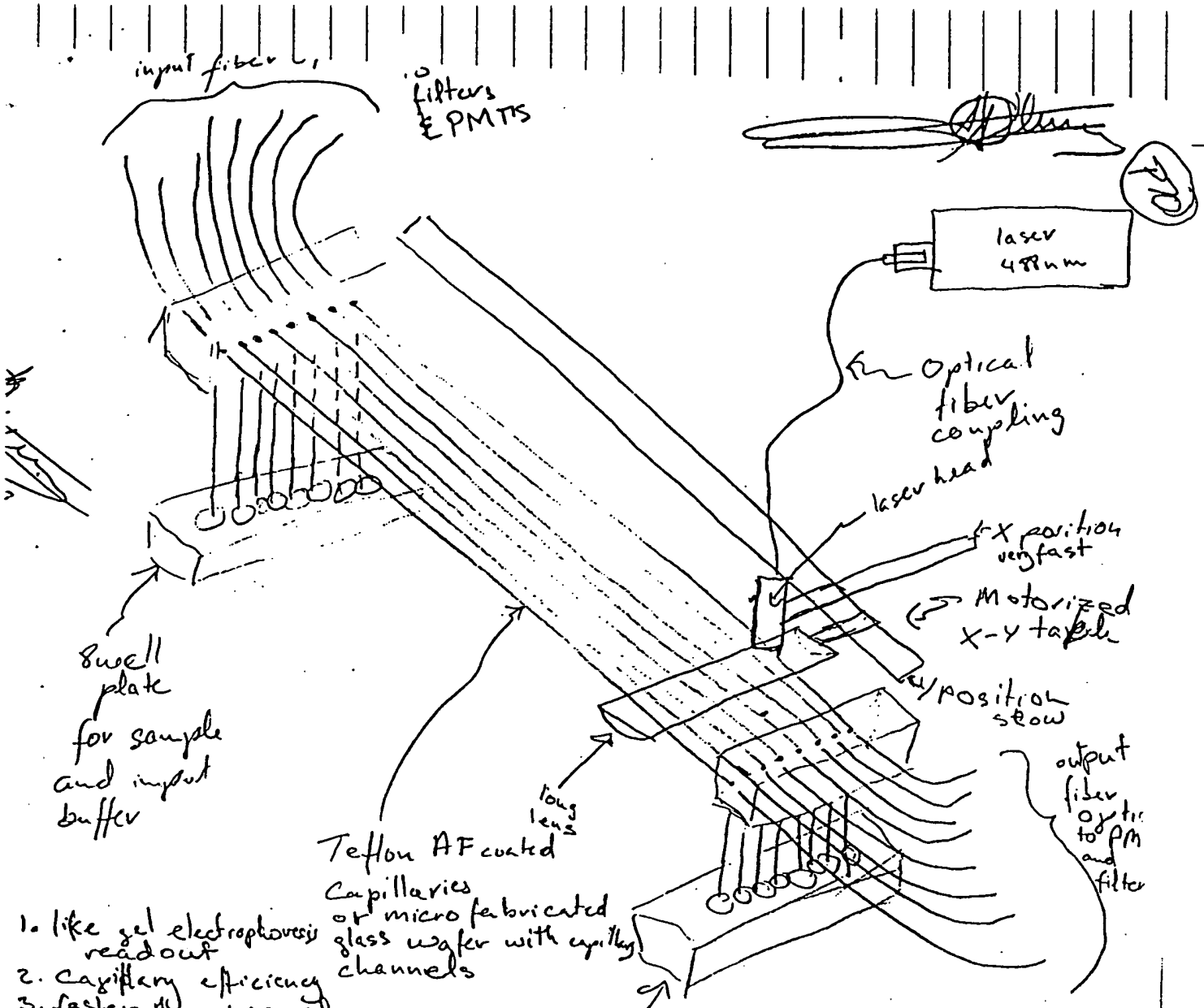
New buffer flushed through the system

10:45 3s inj. sec AB Laser at position 7
No good

11:30 3s inj sec AB Laser at position 3
cap. flushed with 0.1N NaOH water & buffer
peaks at around 1000 s shown AB
degradation and weak signal

12:00 pm 4s inj. sec AB in Tris buffer only
cap. flushed with buffer
Nothing after 1800 s.

1255 pm optimized on an old EWAB solution 4 sec inj
showing peaks at 800 s. with laser at
position 5
file 1008994



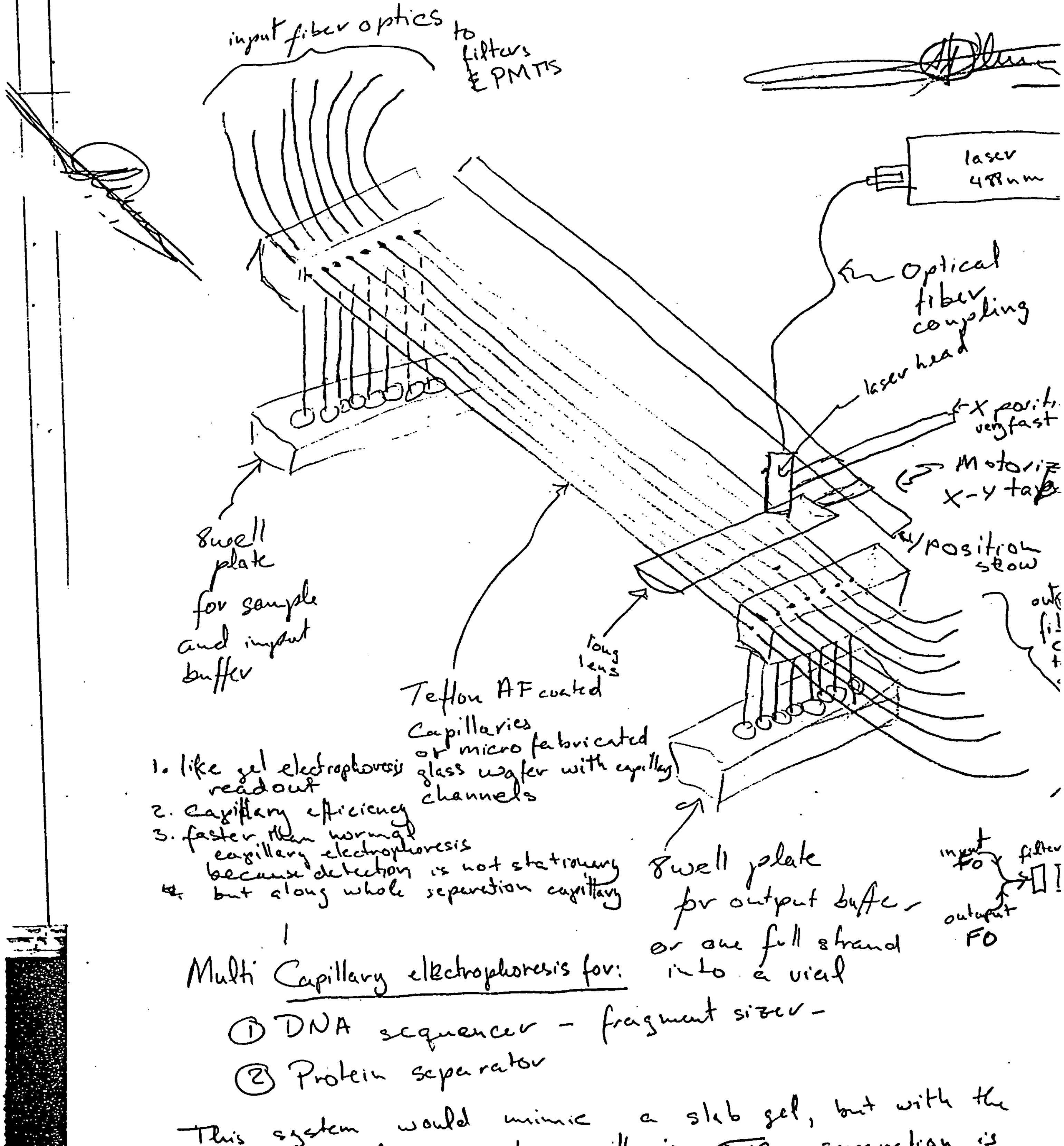
1. like gel electrophoresis readout
2. capillary efficiency
3. faster than normal capillary electrophoresis because detection is not stationary but along whole separation capillary

Multi Capillary electrophoresis for:

① DNA sequencer - fragment sizer -

② Protein separator

This system would mimic a slab gel, but with the separation efficiency of capillaries. ① The separation is carried out until the first signal reaches a preset y position ② Then the separation voltage is decreased and ③ the separated molecules are detected by moving the laser head along the capillaries slowly in the y direction and fast in the x direction ④ Read out could be made a multiple of times, and ⑤ separation continued again to separate unresolve components



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This system would mimic a slab gel, but with the separation efficiency of capillaries. ① The separation is carried out until the first signal reaches a preset Y pos ② Then the separation voltage is decreased and ③ the separated ... detected by ~~slowly~~ moving the laser head along

TRANSMITTAL OF INVENTION DISCLOSURE

DOE Docket No.	Prime Contractor
S-94,638	The Regents of the University of California
Prime Contractor Docket No.	**Prime Contract**
LAD-99-109	W-7405-ENG-36
U.S. Serial No.	**Filing Date**
U.S. Pat.	**Issued (Date)**
Foreign Filing Status	**Industrial Partner**

DOE B&R Code:

KP110202

Source of Funding:

Title

PROTEIN AND DNA FRAGMENT SIZER AND SEQUENCER

Inventor(s):	Employed by:
1. Jose A. Olivares	University of California/Los Alamos National Laboratory
2. Peter Stark	University of California/Los Alamos National Laboratory

Disposition:

- ☐ Contractor intends to elect or request to retain title, and will file a patent application on the invention.
- ☐ Contractor does not intend to elect title. DOE should determine whether to authorize filing of a patent application.
- ☐ Contractor recommends that DOE authorize filing a patent application.
- ☐ Contractor decision will be forwarded at a later date.
- ☐ Contractor does not intend to retain title, and is inactivating this case at this time, because of:
  - ☐ A statutory bar
  - ☐ Other defensive publication
  - ☐ Insufficient patent novelty
  - ☐ Insufficient information for a patent application
  - ☐ Other

Forwarded by:	Date:
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Sue Potter, LC/BPL
File

INVENTION DISCLOSURE

CASE NO.	:	S-94,638
CONTRACT NO.	:	W-7405-ENG-36
LOS ALAMOS DISCLOSURE NO.	:	LAD-99-109
ATTORNEY	:	Samuel M. Freund
TITLE	:	PROTEIN AND DNA FRAGMENT SIZER AND SEQUENCER
INVENTORS	:	Jose A. Olivares and Peter Stark

ABSTRACT :

Use of multi-capillary electrophoresis technology for inexpensive, sensitive and high-throughput DNA fragment and chemical sizing is described. The present invention is also useful for multi-color DNA sequencing applications. A sample to be analyzed is introduced into one end of an electrophoresis capillary, and a voltage is applied longitudinally along the capillary wall which results in electrophoretic separation of the component materials in the sample. The capillary also functions as a liquid-core waveguide. Detection of fluorescence resulting from the interaction of light introduced through the capillary wall with the component materials is then accomplished at the end of the capillary. Excitation of the separated sample components in the capillary may be achieved at any point in the capillary, which permits the instantaneous visualization of the entire electrophoregram. By controlling component movement through the capillary, samples can be removed for further analysis using mass spectrometry or DNA sequencing. The technology will allow analyses to be conducted in a field laboratory.

BACKGROUND :

Amplified Fragment Length Polymorphism (AFLP) and strain-specific polymerase chain reaction (PCR) analyses are the methods of choice for determining the identity of microbes. These procedures provide significantly more information than standard DNA analysis and are more rapid and less expensive than extensive DNA sequencing. AFLP analysis can be used for rapidly characterizing unknown pathogenic species and strains, thereby providing valuable information for developing a therapeutic response to an outbreak thereof. Strain-specific PCR analysis can rapidly identify a previously studied threat species, even if the sample is present in a complex sample mixture. Both methods require further analysis of the reaction results to determine the size of the DNA fragments. Simple detection of the presence or absence of the fragment is insufficient. The time required to complete the reaction analysis is presently between 15 and 20 minutes. However, size analysis is currently conducted using an automated DNA sequencer. The gel procedure requires approximately three hours to complete, plus additional time to set up the sequencing unit and download the resulting data. When faced with the potential or actual release of a biothreat agent, it is important to obtain the genetic information about the released organism in a significantly shorter time period. It is also important to be able to conduct analyses in a field laboratory using affordable apparatus. What is needed is a rapid and low cost method for separating and analyzing small (100 - 500 bp) DNA fragments to precisely determine their size (to within 1 bp). Flow cytometry analysis does not allow resolution of such small fragments. Commercial capillary electrophoresis sequencers have sufficient resolution, but cost approximately $350,000 and are not suitable for rapid fragment size determination.

RELATED ART :

Currently, DNA size analysis requires approximately three hours to run on a gel,

plus additional time to set up the sequencing unit and download the data. Flow cytometry analysis does not resolve 100 to 500 basepair (bp) fragments with 1 bp resolution which is required. Commercial capillary electrophoresis sequencers that can resolve 1 bp cost approximately $350,000 and do not permit rapid fragment size determination or recovery of DNA fragments. Analysis of chemical compositions using capillary electrophoresis has been reported, but general methodologies for introduction and detection thereof are under development.

DESCRIPTION :

The present invention uses multi-capillary electrophoresis technology for inexpensive, sensitive, and high-throughput DNA fragment and chemical sizing. The invention is also useful for multi-color DNA sequencing applications. A sample to be analyzed is introduced into one end of an electrophoresis capillary and a voltage is applied longitudinally along the capillary wall, which results in electrophoretic separation of the component materials in the sample. The capillary also functions as a liquid-core waveguide. Detection of fluorescence resulting from the interaction of light introduced through the capillary wall with component materials of the sample is then achieved at the end of the capillary. Excitation of the separated sample components in the capillary may be achieved at any point in the capillary. Separation is allowed to proceed until the first analyte reaches the prepositioned laser light. Then the separation is stopped, and the excitation laser is rastered along the capillary length to locate and identify the separated components. This process can be carried out multiple times, starting and stopping the separation process as required to separate unresolved components or to remove separated samples for further analysis by, say, mass spectrometry. Use of a plurality of capillaries gives similar results to slab gel technology, but takes only a fraction of the time and yields greatly improved resolution. The present invention should also be faster than conventional capillary electrophoresis DNA sequencers, since the applied voltage does not need to be applied until the last fragment reaches the detector; rather, the detector is moved to the position at which the first separated component appears. The invention permits either the use of separate photomultiplier detectors for each capillary, or one photomultiplier which is rastered across multiple capillaries, thereby eliminating the expensive and insensitive CCD cameras used in conventional DNA sequencers.

REPORTS (including any statutory bar date): None to date.

PROBABLE VALUE : To be determined.